REMARKS

I. Claim Amendments

By the foregoing amendments to the claims, claims 1-4, 9-11, 14, 16-20, 35, 60-62, 64, and 65 have been amended, and claims 6-8, 57-59, and 63 have been canceled.

In particular, the claims have been amended to recite "*fibroblast* feeder cells" instead of "feeder cells." Support for this amendment can be found throughout the specification and claims as filed, such as in the Examples.

In addition, the preambles of claims 1-4 have been amended to recite methods for obtaining "stable" pluripotent human blastocyst-derived stem cell lines. This amendment is supported at least at page 7, lines 24-28 of the specification.

Claims 1-4 have been further amended such that step iii) recites "isolating the inner cell mass-derived cells by manual dissection *into pieces*." Support for this amendment can be found at least at page 14, line 1; and page 18, lines 22-23 and 26-27.

Claims 1-4 have been even further amended to include a step v) for propagating the blastocyst-derived stem cell colonies of step iv), by incorporating, *inter alia*, the subject matter of claims 7 and 8. This amendment is supported at least at page 5, lines 23-25; and page 18, lines 26-27.

Claims 65 and 65 have been amended to depend from claim 62 rather than from claim 63.

The amendments to the claims, including cancellation of claims, have been made without prejudice or disclaimer to any subject matter canceled or recited herein. Applicants reserve the right to file one or more continuation and/or divisional applications directed to any canceled subject matter. No new matter has been added, and entry of the foregoing amendments of the above-identified application is respectfully requested.

II. Claim Rejections Under 35 U.S.C. § 112, First Paragraph

At pages 3-5 of the Office Action, claims 1-20, 35, and 57-65 have been rejected under 35 U.S.C. § 112, first paragraph, as allegedly failing to comply with the enablement requirement.

Specifically, the Examiner has acknowledged that the specification enables obtaining pluripotent human blastocyst-derived stem cells wherein the inner cell mass cells are co-cultured on fibroblast feeder cells, and the blastocyst-derived stem cell line is propagated in

fibroblast feeder cells. However, it is the Examiner's position that the specification does not enable culturing inner cell mass cells or blastocyst-derived stem cell lines on feeder cells other than fibroblast feeder cells.

To expedite prosecution in the present application, and not to acquiesce to the Examiner's rejection, the claims have been amended as described above. In particular, the claims have been amended to recite that the feeder cells are fibroblast feeder cells.

Accordingly, Applicants respectfully request reconsideration and withdrawal of this rejection.

III. Response to Claim Rejections Under 35 U.S.C. § 103

A. At pages 7-12 of the Office Action, claims 1-3, 5-7, 12, 13, 16-20 and 57-59 and newly added claims 62, 63 and 65 have been rejected under 35 U.S.C. § 103(a) as purportedly being unpatentable over Thomson (2001) when taken with Thomson (1998) as evidenced by Stem Information (National Institutes of Health) when taken with Rijinders et al. and in further view of Lanzendorf et al. This rejection is respectfully traversed.

The present claims provide methods for obtaining stable pluripotent human embryonic stem cell lines. The issue of "stability" is discussed in some detail in the application (*see, e.g.*, page 7, line 24, to page 9, line 2). Based on the application, a person of ordinary skill in the art would have immediately recognized that a "stable" pluripotent human embryonic stem cell line is a stem cell line that remains undifferentiated and exhibits no chromosomal instabilities for a period of more than twenty-one months. Applicants note that the stability of the inventive cell line allows for the generation of substantial numbers of cells which may be produced in "lots," each lot comprising a number of individual units (*e.g.* plates, straws, or the like) of similar cells. A skilled artisan would have understood that not only is the generation of large numbers of stable, undifferentiated cells attractive from a commercial point of view, but the ability to produce such large numbers of cells in lots is especially advantageous, because an individual unit may be withdrawn from each lot for characterization of the remaining units within the lot. Such characterization of the undifferentiated embryonic stem cells is desirable from a scientific perspective, enabling the end-user of the cells to know much more about the cells.

Using the method recited in the present claims, the Assignee was able to produce some 3,263 culture units in 2003; 3,578 culture units in 2004; 3,833 culture units in 2005;

8,196 culture units in 2006; and 11,071 culture units in 2007; each lot comprising 100 frozen straws. This is a very significant achievement, and indeed Applicants are unaware of any other entity in the world that is presently able to produce such large quantities of undifferentiated human embryonic stem cells free from chromosomal instabilities. In this connection, the Examiner is respectfully referred to paragraph 8 of the Declaration Under 37 C.F.R. § 1.132 by Dr. Henrik Semb (the Declaration), submitted herewith.

The present claims recite a step of co-culturing the human blastocyst with fibroblast feeder cells to establish one or more colonies of inner cell mass cells (*see* claim 1, step ii)). Applicants submit that this step is not taught or suggested by the references cited by the Examiner.

Further, the present claims recite that the inner cell mass cells are isolated by mechanical dissection into pieces (*see* claim 1, step iii)). The sense in which the term "dissect" is used in the present application is its conventional dictionary meaning, which is "to cut into pieces. " The application as filed (*see, e.g.,* page 14, line 1; and page 18, lines 22-23 and 28-29 of the specification) discloses that such dissection may be performed using a knife or glass capillary, but other methods would have been readily apparent to those skilled in the art. Therefore, in the present methods the colonies are dissected into pieces, and not "pipetted" or otherwise dissociated or disaggregated into separate individual cells or small clumps of cells (*see, e.g.,* paragraph 10 of the Declaration for more detail).

In addition, the present claims recite that the blastocyst-derived stem cells are passaged every four to five days (*see* claim 1, step v)). As discussed on, for example, at page 5, lines 23-30 of the specification, it is important that the cell line is not cultured longer than 4-5 days before passage, otherwise there is an increased probability that the cells will differentiate (*see, also,* paragraph 13 of the Declaration).

The present inventors have surprisingly found that by combining the steps of manual dissection followed by repeated passaging every four to five days, a stable pluripotent human stem cell line can be obtained. Applicants submit that even though the individual method steps may appear to comprise relatively small modifications, the combined effect of such apparently small changes are unexpectedly substantial, allowing for the establishment of highly stable human embryonic stem cell lines which, in turn, allows for the production of significant numbers of cells in well-characterized lots, as discussed above.

According to the Examiner, Applicants' arguments in traversal of the obviousness rejection over Thomson (2001), Thomson (1998), Lazendorf et al., the NIH Stem cell information and Rijnders et al. in the response to the first Office Action were not persuasive.

The Declaration submitted herewith further supports the arguments provided in the prior response. As indicated in the Declaration and discussed below, analysis of the skill in the art, the scope and content of the art, and the difference between the prior art and the present invention leads to a conclusion of non-obviousness.

The level of ordinary skill in the pertinent art

The art at the time of filing was in a state of chaos. While the establishment of murine embryonic stem cell lines had been well documented for many years (since 1981), it had not been possible to establish corresponding lines of human embryonic stem cells. In particular, it was not even known if it would be possible to establish such a stable line of human embryonic stem cells. Numerous different methods have been attempted of course, but as mentioned in the Declaration (*see* paragraphs 16 and 17), the scientific literature on this subject should generally be treated with caution, since negative or adverse results were less commonly published, if at all, and the cell lines were not followed for a sufficiently long period of time to establish their true stability, especially chromosomal stability.

Further, while such papers typically report data pertaining to the expression of markers characteristic of pluripotent cells, they seldom, if ever, report data pertaining to the expression of markers associated with differentiation. Thus while there were reports purporting to describe the establishment of human embryonic stem cell lines, in fact, as confirmed by the Declaration, when attempts were made to reproduce the reported methods, it was found that the cells would ultimately die or differentiate (*i.e.* the cells did not possess sufficient stability), and thus could not be produced in sufficiently large numbers for commercial exploitation. Prior to the present invention, no one had demonstrated the successful establishment of a stable human embryonic stem cell line that remained undifferentiated and exhibited chromosomal stability for an extended period of time (*i.e.* at least twenty-one months, as set forth in the present application). Therefore there remained a major unsolved problem in the field.

The scope and content of the prior art and the difference between the prior art and the present invention

Thomson (2001) discloses the establishment of embryonic stem cell lines (only exemplified by use of embryos from the Rhesus Macaque Monkey). The experimental protocol is described in column 8, lines 32-60 of the reference. According to this protocol, the ICM of human blastocysts is isolated by immunosurgery using anti-human serum antibody followed by exposure to guinea pig complement. Subsequently, the ICM is cultured on mitotically inactivated mouse embryonic feeder cells. 7-21 days after initial plating the ICM-like clumps are removed mechanically by micropipetting and replated on fresh feeder layer. The resulting colonies are passaged every 1-2 weeks.

Thomson (1998) discloses a method for producing embryonic stem cells from human blastocysts. The experimental protocol is described in note 6, page 1147 of the reference. According to this protocol, the ICM of human blastocyst is isolated by immunosurgery using rabbit antiserum. Subsequently, the ICM is cultured on irradiated mouse embryonic feeder cells. 9-15 days after initial plating the ICM-like clumps are removed mechanically by either micropipetting, by exposure to dispase or by exposure to Ca2+/Mg2+-free phosphate buffered saline with 1mM EDTA and replated on fresh feeder layer. Individual colonies with a uniform undifferentiated morphology were individually selected by micropipette, mechanically dissociated into clumps and replated. This reference is silent about the passaging period of the blastocyst derived stem cells.

The experimental protocols of Thomson (2001) and Thomson (1998) thus differ from the methods of the present invention in a number of important respects. First, as noted in the prior response (*see* page 18 of the Amendment and Reply filed July 12, 2007), a person of ordinary skill in the art would not have reasonably predicted that a method established for isolating and maintaining a culture of pluripotent blastocyst-derived stem cells for Rhesus Macaque Monkey would succeed when applied to human cells. Second, in the methods claimed in the present application the colonies of ICM-derived cells and colonies of blastocyst-derived stem cells are mechanically dissected, *e.g.* with a knife or glass capillary, as opposed to being mechanically dissociated by micropipette. Mechanical dissociation by micropipette implies dissociation of the cells into smaller clumps, or even individuals cells, as compared with mechanical dissection of such colonies into pieces as per the present invention which is a more "macroscopic" (in relative terms) procedure. Third, whereas the present invention requires passaging the blastocyst-derived stem cells every four to five days,

Thomson (2001) mention a passaging period of 1-2 weeks, and Thomson (1998) is silent about the passaging period.

The Examiner has also cited Lazendorf et al. (2001). This reference discloses a method for producing embryonic stem cells from human blastocysts. The experimental protocol is described on page 134, under the heading "Immunosurgery", whereas the further differentiation and propagation of the stem cells can be found on page 134 under the heading "Differentiation and Propagation of Human Cell Lines". According to this protocol, the ICM of human blastocysts is isolated by immunosurgery using rabbit antiserum followed by exposure to guinea pig complement, followed by pipetting through a finely drawn Pasteur pipette. Subsequently, the ICM is cultured on irradiated mouse embryonic feeder cells. 4-11 days after initial plating the ICM-like clumps are removed by mechanically disassociation and replated on fresh feeder layer. Individual colonies with a uniform undifferentiated morphology were individually selected and passaged every 3 to 7 days.

As with Thomson (2001) and Thomson (1998), the method described by Lazendof et al. differs from the present invention in that it specifies mechanical dissociation of the ICM cells into clumps comprising small numbers of cells using a finely drawn Pasteur pipette. In contrast to the cited references, the methods recited in the present claims requires mechanical dissection into pieces, which would inherently comprise greater numbers of cells.

In the second Office Action dated October 17, 2007, the Examiner has stated that Lazendorf et al. teaches mechanical disassociation, which according to the Examiner fulfills the limitation recited in the present claims. However, as explained above, the mechanical disassociation described in Lazendorf et al. is not used for isolating ICM cells from the other types of blastocyst cells, but rather uses immunosurgery and pipetting. Thus, Lazendorf et al. neither teaches nor suggests the use of mechanical dissection in a step corresponding to step iii) of the present invention.

Further, whereas the present invention requires passaging the blastocyst-derived stem cells every four to five days, Lazendorf et al. mentions a passaging period of 3-7 days. Even though Lazendorf et al. teaches that the blastocyst-derived stem cells should be passaged every 3-7 days, a stable cell line cannot be established when using a passaging period of, *e.g.*, seven days (*see* paragraph 16 of the Declaration).

Thus the method of present invention differs from Thomson (2001), Thomson (1998) and Lazendorf et al. in at least the following ways: 1) the present claims require mechanical

dissection instead of immunosurgery and/or pipetting to isolate the ICM cells from the other cell types from the blastocyst, whereby larger amounts of ICM cells are passaged, instead of small cell clumps or single cells; and 2) the present claims also require that the blastocyst-derived stem cell colonies are thereafter passaged for 4-5 days, and not 1-2 weeks as in Thomson (2001), or 3-7 days as in Lazendorf et al.

Furthermore, when Thomson (2001), Thomson (1998) and Lazendorf et al. are taken together, it is respectfully submitted that a person skilled in the art would not arrive at the present invention. Firstly, none of the cited references teach or even suggest the use of manual dissection, and secondly, none of the cited references teach or even suggest propagation of the blastocyst derived stem cells for 4-5 days. Lazendorf et al. suggest 3-7 days and Thomson (2001) suggest 1-2 weeks. Taken together, an adaptation to 4-5 days would not have been obvious for a skilled person. Rather a person skilled in the art that attempts to produce large number of cells would attempt to extend the passaging step for as long as possible, since it is in between each passaging step that the cells grow. It would have been counterintuitive to the person skilled in the art, and entirely unexpected, that a larger number of cells could be produced by shortening the passaging period.

Another factor that must be considered in relation to the passaging period is the ease of handling of the cells themselves. The person skilled in the art would be aware that as colonies of cells grow, the colonies become thicker and therefore easier to handle. As discussed here, and in the Declaration (paragraphs 10 and 12), the present invention involves manually dissecting the colonies of ICM-derived cells and blastocyst-derived cells at each passaging step into pieces. With a shorter passaging period, the cells would be less easily handled, with the possibility that they would be impossible to dissect into pieces which could then be lifted off the feeder cells and replated on fresh feeder cells. It is submitted that in addition to the desire to expand the colonies of cells as much as possible, the person skilled in the art would also try longer passaging periods in order to make the pieces of cells dissected from the colonies easier to handle. It is further submitted that the person skilled in the art would be dissuaded from considering shorter passaging periods. Further, shorter passaging periods are more labor intensive and, in a commercial setting, therefore more expensive in terms of human resources.

In view of the foregoing, it is Applicant's position that in addition to the fact that the apparently small modifications made to the method steps and the specific combination of

such steps results in a significant and unexpected solution to a long-standing problem, namely that of providing large numbers of stable pluripotent human embryonic stem cells, the prior art cited by the Examiner fails to provide any teaching that would lead the person skilled in the art to suppose that it was possible to produce a stable human embryonic stem cell line that was capable of remaining undifferentiated for more than twenty-one months without chromosomal instability, or that such stable human embryonic stem cell lines could be produced using the combination of method steps recited in the present claims.

Further, Thomson (2001), Thomson (1998), and Lazendorf et al. fail to report the establishment of a stable human embryonic stem cell line that remains undifferentiated and chromosomally stable for more than twenty-one months. In fact, as noted in the Declaration, as of the priority date of the present application none of the available methods allowed the establishment of human embryonic stem cell lines that are sufficient stable (as defined in the present specification at least at page 7, line 24, to page 8, line 19).

The Examiner cites the NIH stem cell information for showing that the stem cell line produced by Thomson (1998) has a proliferative capacity for more than 21 months. However, the proliferation capacity is only one of several general indications of having a pluripotent and stable stem cell line. As mentioned in the specification on page 7, line 30 to page 8, line 8, these requirements are: normal euploid karyotype, maintaining the potential to develop into all types of germ layers, expression of certain markers, maintaining the pluripotency and capability of differentiating. Therefore, even though the NIH stem cell information states that the cell line produced by Thomson (1998) has a proliferative capacity for more than 21 months, this is not evidence that the stem cell line is stable.

When considering the issue of inventive step, one has to be careful to avoid viewing the invention *ex post facto*. As discussed above, and in the Declaration, at the priority date of the present invention, even though Thomson (2001), Thomson (1998) and Lazendorf et al. were available to the person skilled in the art, these references did not teach or suggest methods for establishing a human embryonic stem cell line with sufficient stability (especially chromosomal stability) to allow the production of large numbers of cells. Such large-scale production was enabled for the first time by the present invention.

As noted above, while the present methods may appear to differ from Thomson (2001), Thomson (1998) and Lazendorf et al. by relatively small modifications to the individual method steps, in fact the combined effect of such apparently small changes in such

method steps is suprisingly substantial, allowing for the establishment of highly stable human embryonic stem cell lines which, in turn, allows for the production of significant numbers of cells in well-characterized lots.

Applicants submit that the benefits of the present methods could not have been predicted. In particular, the reasons for the significant success of the present invention are difficult if not impossible to explain in scientific terms, and in this connection, the best that can be said is that the inventors serendipitously hit upon a combination of method steps which worked. It would be scientifically and legally unsound at this point in time to attempt to dissect the combination of steps forming the complete method into individual steps to try to determine the individual contribution associated with each. Thus, it would not be possible (at least at present) to determine the "proper function" of each method step separately. Although the present methods are not fully understood scientifically, without all the method steps in concert, the method of the present invention simply would not work (*see* paragraphs 14 and 15 of the Declaration).

For at least the reasons set forth above, Applicants submit that claims 1-3, 5-7, 12, 13, 16-20, 21, and 57-59 are patentable over Thomson (2001), when taken together with Thomson (1998), Rijnders et al., (1998), Lanzendorf et al., (2001), and NIH Stem Cell Information. Accordingly, Applicants respectfully request reconsideration and withdrawal of this rejection.

B. At pages 12-13 of the Office Action, claims 4, 8-10 and 60 and 61 have been rejected under 35 U.S.C. §102(a) as purportedly being unpatentable over Thomson (2001) when taken with Thomson (1998) when taken with Rijinders et al. and in further view of Lanzendorf et al. as applied to claims 1-3, 5-7, 12, 13, 16-20, 57-59 and newly added claims 62, 63 and 65 above, and further in view of Marshall et al. (Methods in Molecular Biology: Isolation and Maintenance of Primate Embryonic Stem Cells 158:11-18, January 2001). This rejection is respectfully traversed.

For the reasons stated above, it is Applicants' position that a combination of Thomson (2001), Thomson (1998), Lazendorf et al. and Rinjders et al. would not lead to the invention as recited in the present claims. Applicants further submit that Marshall et al. fails to remedy the serious deficiencies of Thomson (2001), Thomson (1998), Lazendorf et al. and Rinjders et al.

Marshall et al. describes the establishment of Rhesus Macaque Monkey embryonic stem cell lines. The experimental protocol is described at pages 12-15 of the reference. According to this protocol, the ICM of human blastocysts is isolated by immunosurgery using rabbit anti-rhesus or anti-marmoset spleen cell antiserum followed by exposure to guinea pig complement and pipetting. Subsequently, the ICM is cultured on irradiated mouse embryonic feeder cells. Four to six days after initial plating the ICM-like clumps are removed with Ca^{2+}/Mg^{2+}free PBS with EDTA, pipetting, centrifugation and replated on fresh feeder layer. The resulting colonies of stem cells are passaged by pipetting and replated on feeder cells. The reference is silent about the passaging period of the blastocyst derived stem cells (page 15, 3.4).

Thus the method of present invention differs from Marshall et al. at least in that 1) the present claims recite methods for establishing human rather than monkey stem cell lines; 2) the present claims recite mechanical dissection instead of immunosurgery and/or pipetting to isolate the ICM cells from the other cell types from the blastocyst whereby larger amounts of ICM cells are passaged, instead of small cell clumps or single cells; and 3) the present claims recite that the colonies of blastocyst-derived stem cells are thereafter passaged for 4-5 days, whereas Marshall et al. is silent about the time period.

As discussed above, none of the cited references suggest the use of manual dissection, and secondly, none of the cited references suggest propagation of the blastocyst derived stem cells for 4-5 days. Lazendorf et al. suggests 3-7 days and Thomson (2001) suggests 1-2 weeks; Marshall and Thomson (1998) are silent in this respect. For the reasons stated above, it is submitted that an adaptation to 4-5 days would not be obvious for a skilled person. Rather a person skilled in the art that attempts to produce large number of cells would attempt to extend the passaging step for as long as possible, since it is in between each passaging step the cells grow. It is further submitted that it would be counterintuitive to the person skilled in the art, and entirely unexpected, that a larger number of cells could be produced by shortening the passaging period.

In addition, Marshall et al. does not teach or suggest methods for establishment of a stable embryonic stem cell lines that remain undifferentiated and chromosomally stable for more than twenty-one months.

Accordingly, Applicants respectfully request reconsideration and withdrawal of this rejection.

C. At pages 13-14 of the Office Action, claim 11 has been rejected under 35 U.S.C. § 103(a) as purportedly being unpatentable over Thomson (2001) when taken with Thomson (1998) when taken with Rijinders et al. and in further view of Lanzendorf et al. as applied to claims 1-3, 5-7, 12, 13, 16-20, 57-59 and newly added claims 62, 63 and 65 above, and further in view of Cooper.

This rejection is respectfully traversed for at least the reasons set forth above. Applicants respectfully request reconsideration and withdrawal of this rejection.

D. At pages 14-15 of the Office Action, claims 14-15 have been rejected under 35 U.S.C. § 103(a) as purportedly being unpatentable over Thomson (2001) when taken with Thomson (1998) when taken with Rijinders et al. and in further view of Lanzendorf et al. as applied to claims 1-3, 5-7, 12, 13, 16-20, 57-59 and newly added claims 62, 63 and 65 above, and in further view of Gardner et al. (1998), when taken with Gardner (1999).

This rejection is respectfully traversed for at least the reasons set forth above. Applicants respectfully request reconsideration and withdrawal of this rejection.

E. At pages 15-16 of the Office Action, claim 35 has been rejected under 35 U.S.C. § 103(a) as purportedly being unpatentable over Thomson (U.S. Patent No. 6,200,806) when taken with Stratagene Catalog, 1988, p. 39.

This rejection is respectfully traversed for at least the reasons set forth above. Applicants respectfully request reconsideration and withdrawal of this rejection.

F. At page 16 of the Office Action, claim 64 has been rejected under 35 U.S.C. § 103(a) as purportedly being unpatentable over Thomson (2001) when taken with Thomson (1998) when taken with Rijinders et al. and in further view of Lanzendorf et al. as applied to claims 1-3, 5-7, 12, 13, 16-20, 57-59 and newly added claims 62, 63 and 65 above, and further in view of Xu et al. (Pub. No. US 2002/0072117).

This rejection is respectfully traversed for at least the reasons set forth above. Applicants respectfully request reconsideration and withdrawal of this rejection.

CONCLUSION

In view of the foregoing, further and favorable action in the form of a Notice of Allowance is believed to be next in order. Such action is earnestly solicited.

In the event that there are any questions relating to this Amendment and Reply or the application in general, it would be appreciated if the Examiner would telephone the undersigned attorney so that prosecution of this application may be expedited.

Respectfully submitted,

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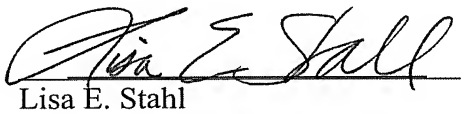
Date: November 17, 2008

By: 
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